Development of an antibody-dependent cellular phagocytosis (ADCP) gene signature to predict prognosis in hepatocellular carcinoma

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Abstract

It remains unclear whether ADCP-related genes are linked to the prognosis of hepatocellular carcinoma (HCC). We obtained RNA-seq data and relevant clinical information on HCC from The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC) databases. We also obtained ADCP-related genes from a previous publication. We developed and validated a five-gene signature (*ELOVL1*, *PRKCD*, *SORD*, *SPN*, and *CBFA2T3*), which was dichotomized based on the median risk score. Patients in the high-risk group exhibited a significantly worse prognosis (*p* < 0.001). To account for other independent prognostic factors, such as the M stage and T stage, we constructed a nomogram that integrated clinical factors and risk scores. The nomogram demonstrated high predictive efficacies of 0.766, 0.784, and 0.797 (AUC) at 1, 3, and 5 years, respectively. Additionally, the low-risk group exhibited increased antitumor immune infiltrates, a higher immune score, and enrichment of antitumor immune pathways. Drug sensitivity analysis revealed that the low-risk group showed higher sensitivity to sorafenib (*p* < 0.001) and rapamycin (*p* < 0.0001) compared to the high-risk group. We identified a five-gene ADCP signature that was correlated with prognosis, immune microenvironment characteristics and drug sensitivity in hepatocellular carcinoma.

Highlights

1. Antibody-dependent cellular phagocytosis (ADCP) is crucial in antiviral immune response and has not yet been reported in the prognosis of hepatocellular carcinoma (HCC).

2. We developed and validated an ADCP-based gene signature containing *ELOVL1*, *PRKCD*, *SORD*, *SPN* and *CBFA2T3* for the first time.

3. The signature significantly correlated with prognosis and the immune microenvironment in HCC.

4. Sensitivity to sorafenib and rapamycin could be distinguished based on the gene signature in HCC.

Introduction

Primary liver cancer (PLC) ranks as the sixth most frequently diagnosed malignancy and the third leading cause of cancer-related deaths worldwide [1]. Hepatocellular carcinoma (HCC) is the predominant form of liver cancer, which accounts for almost 85% of all cases [1]. The most prevalent risk factors for liver cancer include cirrhosis, infection with hepatitis B virus (HBV) or hepatitis C virus (HCV), non-alcoholic steatohepatitis (NASH) associated with diabetes or obesity, and excessive alcohol consumption [2]. Despite significant progress in treatment options, including surgical resection, radiofrequency ablation, arterial embolization, chemotherapy, targeted therapy [3], and immunotherapy [4], HCC remains one of the most challenging cancers to manage, with an overall 5-year survival rate of less than 15% [5]. Accurately predicting prognosis is essential for guiding appropriate treatment decisions for patients.

Antibody-dependent cellular phagocytosis (ADCP) genes play a critical role in the immune response against viral infections [6]. These genes encode proteins that enable immune cells, such as macrophages and dendritic cells, to recognize and engulf cells that have been marked for destruction by antibodies.
Furthermore, ADCP genes stimulate downstream adaptive immune responses by facilitating antigen presentation and stimulating the secretion of inflammatory mediators [7]. Through the process of phagocytosis, immune cells can effectively eliminate virus-infected and cancer cells from the body [8]. Dysregulation of ADCP genes may lead to impaired immune responses and increased susceptibility to viral infections. Although infection with HBV and HCV are the primary risk factors for the development of HCC, the potential impact of ADCP on the genesis and prognosis of HCC remains unclear and warrants further investigation. Therefore, we aimed to investigate whether ADCP-related genes could serve as a prognostic indicator and predict the sensitivity of tumors to specific drugs.

**Results**

**Identification of differential expression of ADCP-related genes**

We used the RNA-seq data of TCGA-LIHC to identify DEGs based on the screening criteria: |Log2FC|>0.58 and P < 0.05 (Fig. 1A) and then extracted the ADCP-related genes, from which we selected 160 significant DEGs for further analysis, including 101 up-regulated and 59 down-regulated genes (Fig. 1B, Table S3). A PPI network was constructed using the String database (Fig. 1C), and key genes were identified using Cytoscape. Correlation analysis was performed on the top 10 key genes to construct a co-expression relationship network (Fig. 1D). We also conducted GO and KEGG functional pathway enrichment analysis on the DEGs, with the significant top 5 pathways being selected for display in GO analysis (Figure S2A), while only 3 pathways were significantly enriched in KEGG analysis (Figure S2B).

**Construction and validation of the ADCP based prognostic signature**

To investigate the prognostic value of ADCP-related genes in HCC, we constructed a gene signature using the TCGA-LIHC cohort. Our workflow for constructing the gene signature is depicted in Supplementary Fig. 1. Firstly, we screened 32 ADCP-related prognostic genes (Table 1) using univariate COX regression. Then we applied LASSO regression analysis on these prognostic genes (Figs. 2A, B) and identified five genes (ELOVL1, PRKCD, SORD, SPN, and CBFA2T3) that were more strongly correlated with prognosis in HCC (Figs. 2C). Among them, lower expression of SORD, SPN, and CBFA2T3 had a worse prognosis while ELOVL1 and PRKCD had a better prognosis in the low expression group (Figure S3). Finally, we constructed a prognostic model consisting of these five genes. The prognostic risk score of each patient was calculated based on the expression of these genes as follows, risk score = ELOVL1* 0.242966292 + SORD*(-0.277111249) + PRKCD* 0.53985067 + SPN*(-0.391114517) + CBFA2T3*(-0.282772196). Based on the median risk score, the training set samples were divided into high-risk and low-risk groups. The Kaplan-Meier curve showed that the prognosis of the high-risk group was significantly worse than that of the low-risk group (Fig. 3A). The ROC curve was used to calculate the AUC values of 1, 3, and 5 years, and they were 0.799, 0.759, and 0.689, respectively (Fig. 3B).
Table 1
32 genes associated with HCC prognosis by univariate Cox regression confirmed

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To validate ADCP-related genes signature, the risk scores of patients in the TCGA-LIHC testing set were calculated using the equation mentioned above, the high-risk group was significantly worse than the low-risk group (Fig. 3C). The AUC for OS at 1, 3, and 5 years was 0.693, 0.696, and 0.701, respectively (Fig. 3D). Meanwhile, our prognostic signature exhibited excellent predictive power in external validation using the ICGC LIHC set. The OS of patients in the high-risk group was significantly lower than in the low-risk group (Fig. 3E). The AUC for OS at 1, 3, and 5 years were 0.802, 0.803, and 0.77, respectively (Fig. 3F). These results suggested ADCP based prognostic signature could serve as a potential prognostic marker in HCC.

The gene signature for nomogram construction

Univariate Cox regression analysis indicated that risk score, T stage and distant metastasis, were independent prognosis indicators in the TCGA-LIHC cohort (Fig. 4A, B). We integrated these three factors to generate a nomogram for predicting prognosis (Fig. 5A), with a C-index of 0.717. Kaplan-Meier survival analysis demonstrated that the OS of the low-risk group was longer than that of the high-risk group (p < 0.0001) (Fig. 5B). The AUC for OS at 1, 3, and 5 years were 0.766, 0.784, and 0.797, respectively (Fig. 5C). The calibration curves at 1, 3, and 5 years exhibited good consistency between actual observed OS and predictive OS by the nomogram (Fig. 5D). Taken together, the nomogram had a good performance on predictive accuracy in the TCGA-LIHC cohort; however, there was no independent external cohort to validate it due to a lack of tumor size data.

Immune landscape of high- and low-risk groups

To investigate whether the risk score value could reflect the state of the tumor immune microenvironment (TIME), we analyzed the association between the ADCP-based prognostic signature and the immunocyte infiltration of the TCGA-LIHC cohort. Firstly, we used the CIBERSORT algorithm to reveal the composition of immune cells. Among the 22 infiltrating immune cells, we found that the low-risk group had a higher infiltration of M1 macrophages and CD4 memory T cells, while the high-risk group had significantly higher expression of M0 macrophages and Treg cells (Fig. 6A). Next, we used the ssGSEA algorithm to discover that the low-risk group had more infiltration of natural killer cells and effector memory CD8-T
cells. However, the high-risk group had a higher distribution of natural killer T cells, activated dendritic cells and activated CD4-T cells (Fig. 6B). We also used the ESTIMATE algorithm on the TCGA-LIHC cohort to find that patients in the low-risk group had a higher immune score, stromal score, and ESTIMATE score (Fig. 6C). Furthermore, we downloaded the immune-related gene set from the IMMPORT database and evaluated the immune pathway scores using the ssGSEA algorithm. The biological differences between the high- and low-risk groups were mainly associated with immune-related pathways. Specifically, pathways of TGFβ family member receptor, cytotoxicity, interleukins, cytokines receptors, chemokines, and BCR signaling were significantly enriched in the low-risk group (Fig. 6D). We also explored the difference in HLA family gene expression and found that the low-risk group had significantly higher expression of HLA-E, HLA-DRB1, HLA-DRA, HLA-DPA1, HLA-DPB1, HLA-DOA, HLA-C, and HLA-B (Fig. 6E).

**Functional enrichment analysis in high and low-risk groups**

To further estimate the biological difference in distinct risk groups, we performed gene set enrichment analysis (GSEA). We found that six important biological pathways were enriched in the high-risk group, including cell cycle, DNA replication, ether lipid metabolism, neuroactive ligand-receptor interaction, nicotine addiction, and ribosome (Fig. 7A). Conversely, the low-risk group was significantly associated with several metabolism pathways (Fig. 7B), including fatty acid degradation, amino acid degradation, tryptophan metabolism, and peroxisome.

**Drug sensitivity analysis in high and low-risk groups**

To select chemotherapy and targeted drugs suitable for patients in the high-risk and low-risk groups respectively, we predicted the IC50 values of common chemotherapy and targeted drugs in TCGA-LIHC based on the GDSC database. IC50 was qualified by the pRRophetic R package. We analyzed the IC50 of 129 drugs and compared the difference in high and low-risk groups. The results demonstrated that the IC50 values of sorafenib (Fig. 8A, Wilcoxon test, p = 0.0002) and rapamycin (Fig. 8B, Wilcoxon test, p = 4.37622E-05) were lower in the low-risk group. More detailed data about drug sensitivity were listed in supplementary table 4.

**Discussion**

HCC is a widely prevalent tumor worldwide, causing increased morbidity and mortality. The available treatment options for HCC are limited, and the prognosis is generally poor. Antibody-dependent cellular phagocytosis (ADCP) genes play a crucial role in the immune response against viral infections, which are the main risk factor for HCC. However, no studies have investigated the correlations between ADCP genes and prognosis in HCC.

In recent years, many gene signatures have been constructed to find the optimal biomarker to predict prognosis and drug response in HCC. Several gene signatures, such as SE-related genes [9], robust
metabolism-related genes [10], pyroptosis-related genes [11], and N6-methyladenosine methylation-related genes [12], have been reported to predict the prognosis of HCC. ADCP-related genes have also been reported to be associated with prognosis in Clear-cell renal cell carcinoma (ccRCC) [13] and to predict recurrence and therapeutic effect in thyroid cancer [14]. Different from previous studies, our research focused on ADCP-related genes and emphasized the important effect of these genes on prognosis, the tumor microenvironment, response to chemotherapy and targeted drugs in HCC.

In this study, we successfully developed and validated a five-gene signature related to ADCP that can predict prognosis and drug sensitivity in HCC for the first time. Among the five genes, sorbitol dehydrogenase (SORD) has been reported to be associated with prognosis in HCC, and high SORD expression in HCC tissues has been associated with favorable effects on OS among patients with liver cancer [15]. Our results were consistent with previous studies. Moreover, we explored the characteristics of TIME and found that the low-risk group had a higher infiltration of anti-tumor immune cells and more pathways involved in regulating immune response, which could contribute to their better prognosis. On the other hand, our results showed that the high-risk group exhibited enrichment in cell proliferation-related pathways, while the low-risk group showed enrichment in metabolism-related pathways. This suggests that the high-risk group may have a more active cell proliferation, while the low-risk group may have a more active metabolism and degradation of various molecules. These findings highlighted the importance of both cell proliferation and metabolism pathways in HCC progression. Additionally, we investigated the relationship between the gene signature and response to chemotherapy and targeted drugs. Sorafenib, which is typically used as a first-line treatment option for unresectable or metastatic HCC, has been shown to increase overall survival (OS) and delay disease progression [16]. Our results suggested that patients in the low-risk group may be more sensitive to Sorafenib and Rapamycin. Mammalian targets of rapamycin inhibitors are associated with reduced rates of hepatocellular carcinoma recurrence after liver transplantation [17]. A combination of Sorafenib and an mTOR inhibitor could be effective in patients with post-liver transplant HCC recurrence not suitable for radical therapy, despite notable toxicity [18]. These results would contribute to developing a personalized treatment plan for patients with HCC.

Our study has several limitations that should be considered. Firstly, our analysis was based on bioinformatics data only, and a more comprehensive understanding of the role of ADCP in HCC requires further in vitro and in vivo studies. Secondly, some of the drug-sensitivity analyses were conducted through simulation, which may not fully reflect real-world outcomes. Lastly, the ADCP-related genes were selected based on a CRISPR screen, and their functions in phagocytosis are not yet fully understood.

**Conclusion**

Our study employed an in-silico analysis that integrated multiple genomic datasets to identify a five-gene signature related to ADCP, which was found to be correlated with prognosis and immune modulation in HCC. Furthermore, our findings showed that the signature-based risk stratification was associated with the response to the targeted drug sorafenib in HCC.
Data & Method

Data collection and genes acquisition

RNA-seq data, the corresponding clinical information (including age, sex, tumor stage, family history, and disease type) and survival information were downloaded from the UCSC Xena platform (https://toil.xenahubs.net/) and ICGC database (https://dcc.icgc.org/). After filtering out the cases with an overall survival time of < 30 days, 343 samples from the TCGA-LIHC cohort and 310 samples from the ICGC cohort were finally included in the subsequent model construction analysis. The detailed clinical information of the two cohorts is shown in Table S1. A total of 543 ADCP-related genes were extracted from previous research [19], of which 511 were found in the TCGA cohort (Table S2).

Construction and validation of the prognostic model

We utilized the R package DESeq2 to analyze the differential expression genes (DEGs) between tumor and normal tissues [20] based on the screening criteria: |Log2FC|>0.58 and P < 0.05. The DEGs-related ADCP were obtained after the intersection of DEGs and ADCP genes. The tumor samples in TCGA-LIHC were randomly allocated into a training set and a test set at a ratio of 6:4. Additionally, the data from ICGC were used as an external validation set. In the training set samples, univariate Cox regression analysis was used to screen genes significantly related to survival prognosis based on DEGs-related ADCP. LASSO Cox regression model of glmnet R package was used for building the Riskscore model as follows [21], Riskscore = \sum \beta_{\text{gene}} \times \text{Exp}_{\text{gene}}. To ensure the accuracy of our model, we utilized the Riskscore calculation formula and applied the same regression coefficient to calculate the Riskscore value for the test set of TCGA-LIHC and the external validation set of ICGC LIHC. We then established the median value of Riskscore as the cutoff point, dividing the samples into high-risk and low-risk groups. We evaluated the association between Risk groups and survival prognosis using the Kaplan-Meier curve method with the R package "survival". Furthermore, we utilized the R package "survival ROC" to plot 1-year, 3-year, and 5-year ROC curves and calculated the corresponding AUC values for each time point.

Immune profile analysis

ESTIMATE, CIBERSORT [22] and ssGSEA [23] were used to calculate the scores of TME scores and immune cells proportion, and the Wilcoxon test was used to evaluate the differences between the risk-high group and risk-low group. The immune genes-related pathways were obtained from the Immport database (https://www.immport.org/home). The ssGSEA was used for calculating the immune pathway score. Wilcoxon test was used to compare the expression differences of HLA family genes and immune pathways between high and low-risk groups.

Gene set enrichment analysis
DESeq2 was used to discover DEGs between the high-risk group and low-risk group, and then all genes were sorted based on log2FC. GSEA analysis based on KEGG pathways was performed using the ClusterProfiler package [24] to find the significantly enriched pathways in high and low-risk groups, respectively.

**Prediction of drug susceptibility**

The susceptibility of chemotherapy and targeted drugs was estimated using the Genomics of Drug Sensitivity in Cancer (GDSC) (https://www.cancerrxgene.org/) database. The half-maximal inhibitory concentration (IC50) was quantified using the pRRophetic package in R [25]. Wilcoxon test was used to compare the differences in drug sensitivity between high and low-risk groups.

**Statistical Analysis**

We utilized t-tests and Wilcoxon tests for continuous variables and chi-square tests for discrete variables. All analyses of this project were completed in R 4.2.1, and a p-value < 0.05 was considered statistically significant.

**Declarations**

**Ethics approval and consent to participate**

RNA-seq data and corresponding clinical information (including age, sex, tumor stage, family history, and disease type) were obtained from public databases.

**Data availability statement**

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

**Author contributions**

YZW and XMK conceived and designed the study. JYW collected the data, performed bioinformatics analysis, and wrote the initial draft of the manuscript. XZ analyzed the data and generated the figures. YZW conceptualized and revised the manuscript. All authors contributed to the article and approved the submitted version.

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None.

**Conflicts of Interest**

The authors declare no conflict of interest.
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None.

References


**Figures**
Figure 1

Differential expressed genes (DEGs) analysis in HCC patients. (A) Volcanic Map of ADCP-related DEGs in TCGA. (B) Venn diagram of ADCP-related target genes in different groups. (C) PPI network diagram of ADCP-related DEGs. (D) Expression correlation network diagram of hub genes in ADCP-related DEGs.
Figure 2

Construction of prognostic model. (A-B) The LASSO Cox regression model was constructed from the ADCP-related DEGs. (C) Relationship between five identified genes and prognosis of HCC.
Figure 3

Construction and validation of the ADCP-based prognostic risk signature. (A) Survival curves for the low-risk and high-risk groups in the TCGA-training cohort; (B) Time-independent ROC analysis of risk score for prediction of the overall survival in the TCGA-training cohort; (C-D) Survival curves and time-independent ROC analysis in the TCGA-testing cohort; (E-F) Survival curves and time-independent ROC analysis in the ICGC validation cohort.
Figure 4

Independent prognostic factor analysis of risk scores and clinical parameters. Univariate (A) and multivariate (B) Cox regression confirmed that the risk score, M stage, and T stage were independent prognostic factors.
Figure 5

A nomogram was constructed to predict the survival of HCC patients in the TCGA cohort. (A) The nomogram for predicting the overall survival of HCC patients at 1, 3, and 5 years. (B) Kaplan–Meier survival curves indicated that the overall survival in the low-risk group was markedly higher than that in the high-risk group. (C) The ROC curves of the nomogram for the survival prediction of HCC patients at 1, 3, and 5 years. (D) The nomogram calibration curves of 1, 3, and 5 years survival probabilities.
Figure 6

Box plot showing the immune profile in HCC patients from high-risk and low-risk subgroups. (A) 22 types of immune cells by cybersport; (B) Cell proportion by ssGSEA; (C) the score by Estimate. (D) Expression of HLA family genes; (E) ssGSEA score of the immune-related pathways. Wilcoxon test was used for data analysis. The p values were shown as *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001, ns for not significant.
Figure 7

KEGG enrichment analysis. (A) Top six pathways in the high-risk group; (B) Top six pathways in the low-risk group.

Figure 8

Boxplot shows the relationship between IC50 and RiskGroup. (A) Sorafenib (p<0.001); (B) Rapamycin (p<0.0001).

Supplementary Files

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